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STUDIES ON THE PARASITISM OF *CERCOSPORELLA NESLIAE*

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Department of Field Crops

University of Alberta
April, 1932

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STUDIES ON THE PARASITISM OF *CERCOSPORELLA NESLIAE* BISBY AND DEARNESS

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Department of Field Crops

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STUDIES ON THE PARASITISM OF CERCOSPORELLA NESLIAE BISBY AND DEARNESS

Eric George Sharvelle

INTRODUCTION

The fungus Cercosporella Nesliae is a pathogene which causes a destructive disease of ball mustard, Neslia paniculata, in western Canada. This pathogene was originally described by Bisby and Dearness (1) from collections made in Manitoba in 1928. Recent surveys in Alberta indicate that the disease caused by this pathogene is one of the most prevalent diseases occurring on any noxious weed in the province. It was on this account chosen for special study.

The object of this investigation has been to study the disease caused by Cercosporella Nesliae including various factors conditioning its development, and to determine whether or not the causal organism is of practical significance in the control of ball mustard, or of importance in attacking plants of economic value.

DISTRIBUTION OF THE DISEASE

The first report of this disease in Alberta was made during the summer of 1930. During the summer of 1931 a weed disease survey was conducted throughout the province and the distribution of Cercosporella Nesliae was determined at this time. The disease was noticed to be particularly prevalent in the north central part of the province, and was reported as far north as Athabasca Landing, and in the vicinities of Colinton, Clyde, Westlock, Pibroch, Dapp, Sangudo and Cherhill. In the vicinity of Edmonton practically 100% infection was observed in all stands of ball mustard, the disease being reported in the localities of Sand Lake, Clover Bar, Wetaskiwin, Breton, Oliver, Morinville, St. Albert and Fort Saskatchewan. The disease was probably more prevalent in these localities than in any other part of the province.

South of Edmonton, as one approaches the drier areas, the frequency of ball mustard plants becomes less, but heavy infection in all stands was noticed. In this region the disease was reported from Red Deer, Donalda, Innisfail and Trochu. South of Innisfail the number of ball mustard plants becomes fewer and fewer until they become quite infrequent. In only one locality were ball mustard plants noticed to be free from the disease, and this was in the neighborhood of Pine Lake, situated east of Innisfail.

The failure of ball mustard plants to occur in any number in the south of the province would seem to indicate that the climatic and soil conditions of southern Alberta are unfavourable to this weed, and in the light of subsequent

investigations it seems probable that should Neslia paniculata occur in this region it would be relatively free from infection by Cercosporella Nesliae.

SYMPTOMS OF THE DISEASE

General

The first indication of the disease in the field is marked by the appearance of well defined circular lesions on the leaves of infected plants. Later, brown, restricted, water-soaked lesions make their appearance on the stems of the plants, while in further advanced stages of the disease, infected plants become lighter in colour, the leaves shrivel and curl up, and the plants present an appearance as if scorched by the hot sun, the term "blight" well describing the appearance of the plants at this stage. In the final stage of the disease the plants become entirely devoid of leaves, while the stems and fruits turn a greyish-black colour.

The appearance of the disease in the field is quite striking as entire patches of ball mustard plants may become affected, the defoliated plants at maturity presenting a characteristic blackened appearance.

On the Leaves

The first symptoms of the disease become evident from two to three weeks after inoculation of the plants in the greenhouse and appear in the form of small, circular, pale-greenish spots which gradually enlarge to form the typical leaf lesions, occurring most commonly at the tips and margins of the leaves (Fig. 1). The lesions appear as roughly circular spots, the colour changing progressively from a light green, to a light grey and ultimately to a dull slate colour as the fructifications make their appearance. The centre of the lesion is usually lighter in colour than the rest of the spot, and frequently, but not always, appears to have shrunken, forming a slight depression, thus sharply dividing the necrotic from the normal tissue. Quite commonly the mature lesions present an indistinct zoned appearance.

Generally, the lesions start near the leaf margins and gradually coalesce until nearly the whole of the leaf blade tissue may become involved. About six weeks after the inoculation of the plants most of the leaves have fallen off and those remaining are in most cases almost covered by the lesions and usually much shrivelled and curled. Upon examination of the mature leaf lesions under the binocular microscope, it is noticed that they are covered with numerous small, shiny-black, irregular bodies which upon closer examination are seen to be without

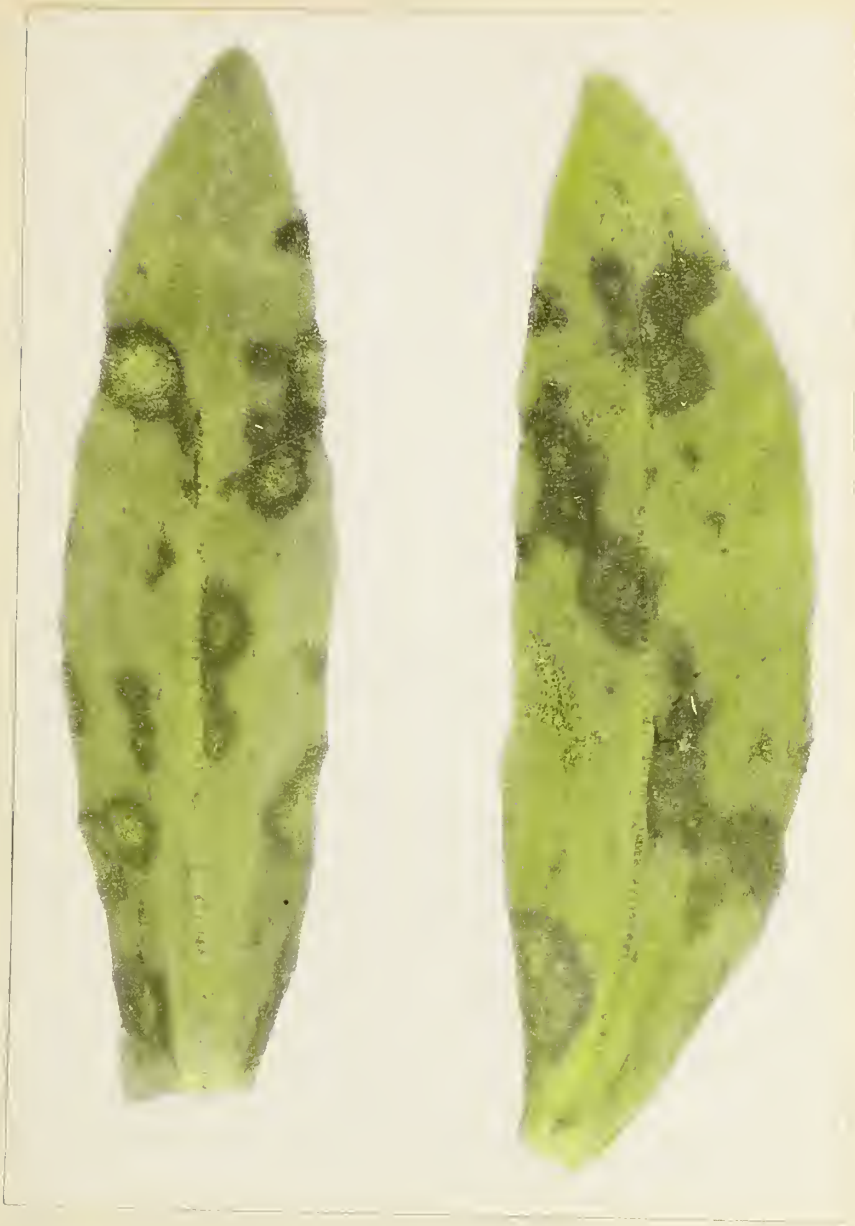

Fig. 1. Early disease symptoms on the leaves of artificially inoculated plants.

definite organization and to consist of a mass of intertwining fungous hyphae which burst through the epidermis to form a stroma on the surface of the leaf. Conidiophores and conidia are produced on the surface of the stroma and these may be separated by floating the leaves on water. Diseased leaves in general are characterized by their lack of turgidity and lightness of colour, as compared with the dark-green, turgid leaves of healthy plants.

On the Stems

From a few days to a week after the appearance of the lesions on the leaves, dark-green to brown coloured lesions make their appearance on the stems. At first the lesions are confined (Fig. 2), but later gradually spread along the stem until the whole stem may become involved. The rate of spread of the lesions appears to be dependent to a large extent upon the environmental conditions, the most rapid spread occurring in relatively humid atmospheres. The colour of the stem lesions changes progressively from dark green, to brown, to grey and finally to slate-grey or almost black; the black colour being due to the abundance of small black fungous bodies protruding through the epidermis, a single one being barely visible to the naked eye. Upon close examination the small black bodies are seen to be irregular black stromata identical with those described under leaf symptoms.

Fig. 2. Early disease symptoms on the stems.

On the Flower Pedicels

About six weeks after the inoculation of the plants, the stem lesions in the course of their development have reached the top of the plant and have spread out along the flower pedicels. The lesions on the pedicels appear to proceed through the same changes as described for the stem lesions, eventually becoming slate-grey with the typical black dots also present (Fig. 3).

On the Fruits

On badly infected plants, the fruits appear to be fewer in number than on healthy plants, and such fruits that are present, exhibit the characteristic blackened appearance of diseased stems and generally are somewhat smaller than fruits from healthy plants.

ETIOLOGY

Isolation of the Organism

The organism was first isolated from diseased stems of Neslia paniculata collected during the provincial survey of 1930. The diseased stems were surface sterilized and plated out on ordinary potato dextrose agar, the resulting growth appearing as a dark-grey to black, confined, slow growing, irregularly circular colony (Fig. 14).

Fig. 3. Disease symptoms on the flower pedicels.

The fungus was noticed to grow particularly well from diseased pedicels, but attempts to isolate the organism from diseased fruits obtained during the survey were unsuccessful at this time. Later, using material artificially inoculated in the greenhouse, it was possible to isolate the organism from diseased leaves, stems, pedicels, and fruits.

Proof of Pathogenicity

During the winter of 1930-31 a number of ball mustard plants in all stages of growth were inoculated in the greenhouse with a suspension of spores obtained from the pathogene in culture. At this time negative results were obtained in the inoculation experiments and it was thought that probably the temperature relationships in the greenhouse might be the causal factor in the failure to obtain infection. With this data in mind a number of plants were inoculated and placed in greenhouses at the three different temperatures, 50-60°F., 70-75°F. and 85°F., again with negative results. Attempts were then made to produce infection by means of stem punctures and the introduction of inoculum into the wound, also with negative results. As a result of these preliminary experiments it was thought that the failure to obtain infection might be due to unfavourable humidity conditions in the greenhouse. In view of these facts, an inoculation

cage was constructed (Fig. 4), consisting of a wooden frame covered with cheese cloth which was kept moistened at all times. The pots of inoculated plants were placed in moist sand on the bottom of the cage.

Fig. 4. Inoculation cage in greenhouse.

The plants to be inoculated were first sprayed with a suspension of spores and mycelium obtained from the pathogene in culture and were then placed immediately in tall metal incubation chambers for a period of forty-eight hours, after which they were removed and placed in the inoculation cage where they remained until disease symptoms were evident.

At the end of three weeks following inoculation definite symptoms of the disease were apparent on the inoculated plants, and "Koch's rules of proof" were followed in establishing the pathogenicity of the organism isolated

from lesions on the stems, leaves, pedicels and from the surface of diseased fruits.

MORPHOLOGY

Mycelium

The mycelium of the pathogene as it occurs within the tissues of the host is composed of septate, hyaline, fairly thick-walled hyphae varying from 3.0-5.0 microns in width. The young hyphae appear as delicate, irregularly septate, thin-walled threads, but with advancing age the number of septa increases, the walls of the hyphal cells thicken, and the cell contents become granular with numerous oil globules present. The older mycelial hyphae are very irregular in shape and the hyphal cell contents become light brown in colour.

In artificial culture the morphology of the mycelial hyphae varies greatly depending upon the culture medium employed. On certain media, (e.g. corn meal agar), the individual cells of the hyphae become densely granular with numerous oil globules, light brown in colour and greatly swollen with stored food giving the mycelial hyphae a distinct bead-like appearance due to the constriction of the cells at the septa.

Conidiophores

The conidiophores emerge from the leaf in distinct fascicles (Fig. 5B). When observed in surface view these fascicles of conidiophores may be seen to emerge through the stomata of the leaf (Fig. 5A), forcing the guard cells apart and completely destroying the stoma as the base of the fascicle enlarges.

At first the conidiophores are hyaline and continuous, but at maturity one to several septa may be formed towards the base of each conidiophore. The contents eventually become granular and numerous oil globules are present. In length the conidiophores vary from 17.2-37.4 microns and vary from 1.5-3.0 microns in width at the base.

The manner of attachment of the spores to the conidiophores could not be traced due to the delicate nature of these structures, the spores in every case being broken off during examination. In this study, diseased leaves bearing a crop of conidiophores were sectioned freehand and stained with aqueous stains. It was found that the use of alcoholic stains, involving subsequent treatment in an alcohol series and mounting in Canada balsam, resulted in distortion and rupture of the delicate conidiophores. Lacto-phenol, cotton-blue stain was found to be ideal for this work, the conidiophores and hyphae being stained deep blue while the leaf tissue remained unstained. Stained preparations of conidiophores were mounted in gum-arabic to avoid the distortion occasioned by the use of an alcohol series and Canada balsam.

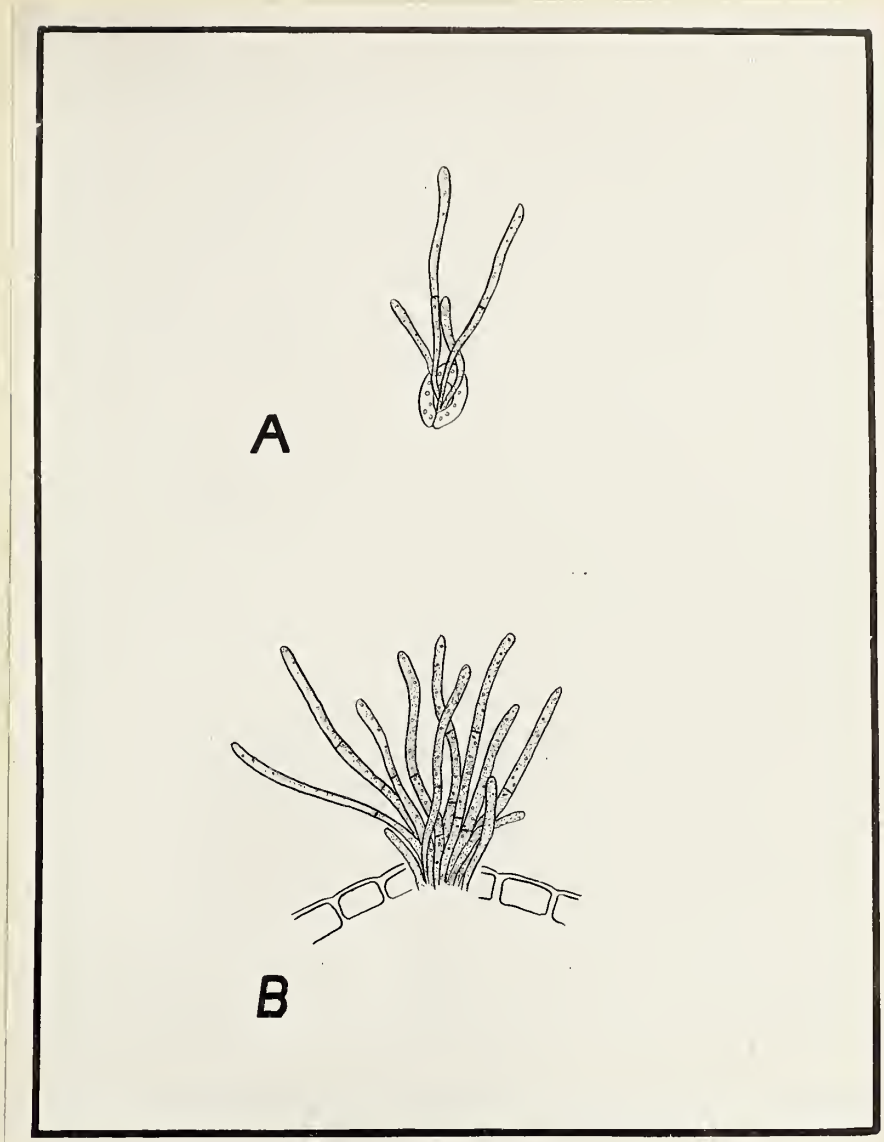

Fig. 5. Conidiophores of Cercosporella Nesliae.

Stromata-like Bodies

In the advanced stages of the disease small, black, stromata-like bodies make their appearance on the surface of the leaf lesions and on the surface of diseased stems and pedicels as described under "Symptomology". The morphology of these structures was studied by sectioning diseased pedicels freehand and staining with Pianese IIIb stain or with acid-fuchsin, light-green stain. With the former stain the host tissues stain green and the mycelium a deep pink, while with the latter stain the host tissues stain blue green and the diseased tissues and hyphae assume a pink to red colour. When mounts were prepared as described above, it was observed that the fungus forms a dense mat of hyphal cells in the outer cortex of the petiole, and the stromata-like bodies, consisting of dense masses of hyphae, rupture the epidermis and become apparent at the surface (Fig. 6).

It would seem probable that these stromata-like bodies are responsible for the initial production of conidiophores and spores in the spring, as when material bearing such structures collected some months previously was incubated under conditions of high humidity, hyaline hyphae were produced from their surface. From a study of the life history of the organism it would seem probable that these hyphae are the developing conidiophores, although spore production was not actually observed from these bodies.

Fig. 6. The development of stromata-like bodies in the outer cortex of the petiole of Neslia paniculata.

Spores and Spore Germination

According to Dearness and Bisby (1) the conidia of Cercosporella Nesliae are described as: "hyaline, straight, seldom curved, mostly continuous even to slightly oboclavate, 30-75 x 2.5-3.5 microns mostly shorter than 50 microns." According to Clements (3) the conidia of the genus Cercosporella are described as "hyaline or bright coloured, 2-several septate, oblong, fusoid or elongate." In this investigation the spores were observed to be hyaline, 1-3 septate, but practically never more than three septate, sometimes curved, obclavate and elongate, thus agreeing with the description of Clements for the genus and disagreeing with the description of Bisby and Dearness. The dimensions of the spores are given in Table 1. It will be seen from this table that the spores range in length from 28.08 - 98.84 microns, the average length being over 55.37 microns.

TABLE 1.

Spore size of Cercosporella Nesliae.

	Minimum	Maximum	Average
Length in microns	28.08	98.84	55.37
Width at base (u)	1.56	4.68	3.12
Width at apex (u)	1.56	3.12	2.34
Number of septa	0	3	2

In the study of spore germination the method of Henrici for the study of molds, yeasts, and actinomycetes was adopted (8). This method briefly outlined is as follows:

A piece of blotting paper, or several thicknesses of paper towelling, are placed in the bottom of a petri dish and the whole is then sterilized in the hot air oven. Just before using the filter paper is moistened with sterile water. A number of cover glasses are cleaned and sterilized by flaming in an alcohol flame and are then placed on the surface of the filter paper in the petri dish. A tube of ordinary potato dextrose agar, previously filtered, is then placed in a hot-water bath and melted, cooled down to 40-50° C., and inoculated rather heavily with the spores of the organism to be studied. With a wire loop one or two loopfuls of the inoculated agar are then placed on the cover glasses and spread in a rather thin film. The petri dish containing the inoculated cover glasses is then placed in the incubator at the desired temperature and the germination traced at different time intervals using the camera lucida. After the cultures have grown sufficiently they may be examined directly mounting the cover glasses on a drop of water on a slide, or they may be made into permanent mounts. In the latter dase several procedures are available depending on the purpose of the slide. For the general morphology of the fungus the following procedure was followed:

After drying the agar film, each cover glass was mounted on a drop of Amann's medium having the following composition:

Phenol crystals	20 grams.
Lactic acid syrup (85%)	20 "
Glycerol	40 "
Water	20 "

these are dissolved together with gentle warmings and then Cotton blue.....0.05 grams is added to the above mixture. This is the formula given by Linder (11) save that the amount of dye is greatly reduced. It serves as a combined fixing agent, stain, and mounting fluid. The mycelium and spores will be stained blue removing much of the dye from the solution. Enough fluid must be used to fill the space under the cover glass without air bubbles. The excess fluid is removed from the edge of the cover glass and the slide may then be sealed, various cements being recommended for this purpose, Canada balsam and gold size being most widely used. In this investigation it was found that gold size was rather inclined to run under the cover glass and was not as satisfactory as Canada balsam.

An especially safe procedure in sealing the mounts is that proposed by Diehl (5). The cover glass is first mounted on a larger cover glass and the latter is then inverted on a drop of balsam on a glass slide. In any case, it is advisable to let the slide stand for several days

before sealing in order to allow the excess fluid to evaporate at the edge of the cover glass.

After the preparation of the slide cultures as described above, they were placed in the incubator at 20° C. and examined under the microscope every 12 hours. The normal spores before germination were observed and drawn with the aid of the camera lucida, as were all the diagrams in this phase of the work. Before germination the spores were observed to be long, linear, hyaline, 1-3 septate with the basal portion often slightly enlarged (Fig. 7A), this probably being the place of attachment of the spore to the conidiophore. Numerous, small, non-septate spores were noticed and it was assumed that these were the younger spores as septations were noticed to develop in later stages.

After 24 hours incubation at 20° C. and previous to the production of germ-tubes, the spores were observed to increase markedly in width and somewhat in length. There does not appear to be any alteration in the internal structure of the spores but they become distinctly constricted at the septations and present a bead-like appearance (Fig. 7B). After 36-48 hours incubation at 20° C. the spores were noticed to produce germ-tubes both laterally and terminally (Fig. 7C). In most cases the germ-tube is thrust out near the septum dividing the cells of the spore and becomes septate after it has reached a certain stage of development (Fig. 7D).

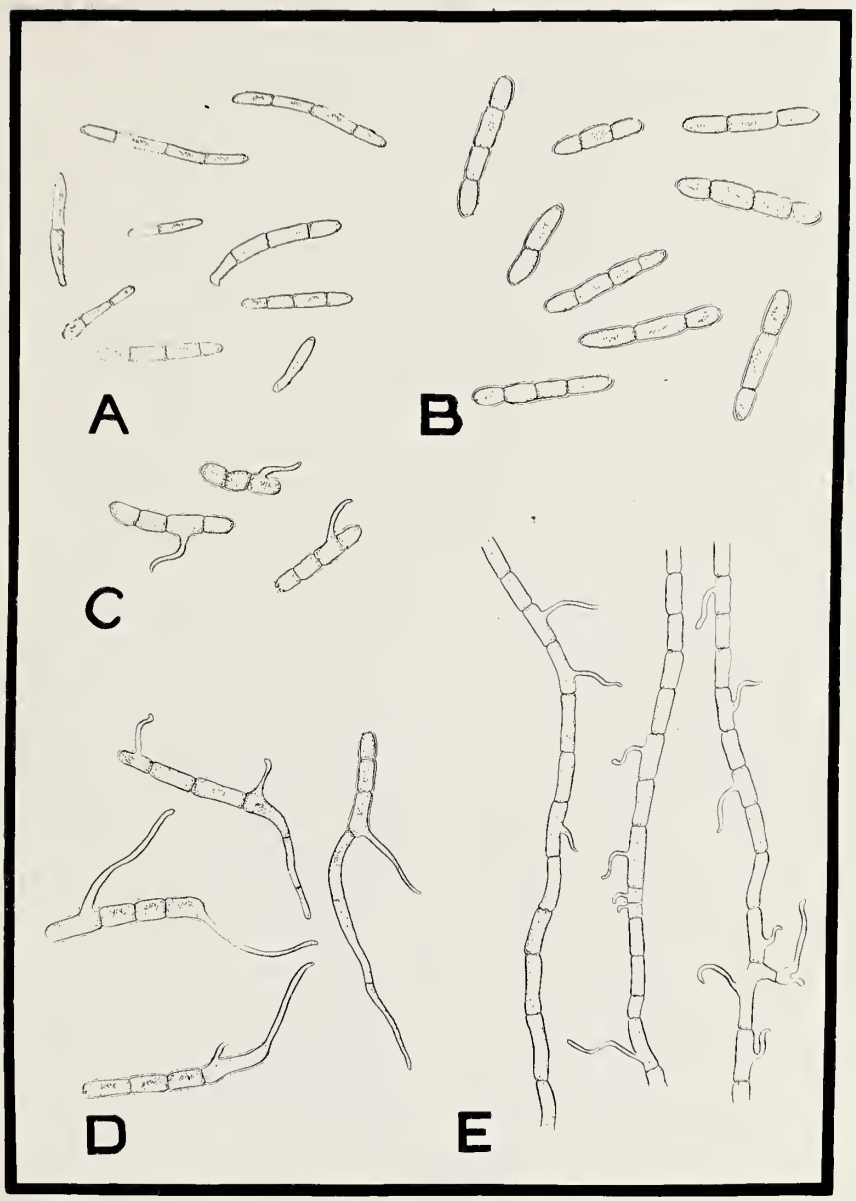

Fig. 7. Mode of germination of spores of Cercosporella Nesliae.

There seems to be no constancy as to the mode of germ-tube production both polar and lateral germination occurring indiscriminately. Following an incubation period of about 72 hours the growth of the germ-tube proceeds. The germ-tubes develop to form hyaline, slightly granular, septate hyphae, but it was not possible to follow the method of spore formation using this method due probably to the thinness of the agar film being unfavourable for spore formation (Fig. 7E).

Taxonomic Position of the Causal Organism

The causal organism was first described in 1928 by Bisby and Dearness (1) under the name of Cercosporella Nesliae. At that time the pathogene was described by the above investigators as occurring on the living leaves of Neslia paniculata and was first collected at Brandon, Manitoba, on July 1, 1928, and later at Foxwarren, Manitoba, on July 19, 1928. Since that time apparently no report of the organism has been made in western Canada and no reference is made to it in Seymour's "Host index of the fungi of North America" (14). Cercosporella is a genus of the Fungi Imperfecti, belonging to the order Moniliales, and the family Moniliaceae.

Life History of the Causal Organism

From a study of Cercosporella Nesliae it seems probable that the pathogene passes the winter in the form

of dormant mycelium within the dead stems, or other diseased parts, of badly infected plants. By late fall, when all plant growth has ceased, infected mustard plants are characterized by their complete lack of leaves and the greyish-black colour of their stems. Microscopic examination of the latter reveals the presence of typical stromata and fascicles of conidiophores bursting through the epidermis and appearing to the naked eye as minute black dots. Close examination of diseased stem material in the fall failed to reveal the presence of spores on the conidiophores, and it seems likely that all spore production had ceased before this time.

Successful inoculations of mustard plants in the greenhouse were made in the fall of 1931 from isolations obtained from material collected in the summer of 1930. It seems probable that with the return of moist conditions in the spring the dormant mycelium awakens to life and produces conidiophores and conidia, as diseased material kept in the laboratory for twelve months immediately produced a crop of spores when placed in humid surroundings.

The fact that the organism grows fairly well on boiled mustard leaves and stems may indicate another means of overwintering and source of inoculum the following spring. The complete life cycle of the pathogene may be outlined as follows: the spore, after reaching the mustard leaf, and under suitable conditions of temperature and humidity, germinates and sends its delicate, thread-like

infection hypha into the leaf tissues by way of the stomata. The fungus grows within the leaf tissues, absorbing nutriment from the leaf cells and producing a well defined spot. After the exhaustion of the nutriment in the centre of the lesion the fungus sends out fruiting hyphae which produce spores capable of immediately infecting adjacent plants, providing environmental conditions are favourable. It is significant that in the spring lesions appear on the leaves only, and several weeks later they make their appearance on the stems.

During the summer of 1931, in the vicinity of Edmonton, the leaf lesions first made their appearance in the field on June 8. Four weeks later, on July 10, the first stem lesions made their appearance in the field, and it is significant that they appeared following a period of fairly heavy rainfall. This would seem to suggest that the stem lesions appear as the result of secondary infections established by conidia produced from the surface of the leaf lesions, the stems apparently being resistant at the time of primary infection and the establishment of the disease within the leaves.

This course continues throughout the warm, humid weather of late spring and early summer until the coming of cool-rainy weather in the fall, secondary infections probably being established by conidia from time to time throughout the growing season. In the fall, with the coming of cool, rainy weather, the fungus within the diseased

plants apparently assumes a resting stage in the form of stroma-like masses of heavy-walled mycelial threads and dormant hyphae. In this manner the fungus continues its existence through the winter until the arrival of warm, humid conditions when it once more commences its life cycle.

It is significant that material which had been allowed to remain in field throughout the winter of 1931-32 readily produced conidiophores and spores when brought into the laboratory on March 1 and incubated in a moist chamber.

RELATION OF THE PARASITE TO THE HOST

Mode of Penetration of the Host

The method of penetration of the organism into the suscept was demonstrated by placing mature leaves of ball mustard in a moist chamber and inoculating with mycelium and spores from diseased leaf material. The diseased leaf material was collected from the field during July and was placed in a moist chamber for 24 hours. After this period of incubation the leaves became covered with a greyish felt of conidiophores and conidia. A suspension of spores and mycelium was then made in sterile water and sprayed over the surface of the leaves in the moist chamber. Pieces of leaf epidermis were then stripped off from time to time and examined for penetration. Permanent

mounts were then made in Amann's medium and in lacto-phenol mounting fluid.

In these studies it was noticed that the fungus enters the leaf most readily when conditions of high humidity prevail in the surrounding atmosphere. However, where the water forms a visible film over the leaf surface the germ-tubes do not penetrate the stomata.

About 24 hours after the inoculation of the leaves in the moist chamber with a suspension of spores, examination of pieces of epidermis stripped from the upper surface of the leaves showed the production of germ-tubes from the spores lying on the surface of the leaves (Fig. 8A). It was noticed that there was a tendency for the spores to produce germ-tubes from the portion of the spores nearest the stoma; when the spore is lying at right angles to the stoma polar germination takes place, while when the spore is lying parallel with the stoma lateral germination occurs. There appears to be a tendency for the germ tubes of the spores to grow towards the stomata of the ball mustard leaf. Klotz (10) studying the early blight fungus of celery, Cercospora apii Fres., states that:-

> "it was noted with spores germinating upon a glass surface that where a moisture film enveloped the germ tube, this film was pulled along with the extending tube. This small film enveloping the spores may be significant in the infection process. It is likely that the tube extends the enveloping water film until the film is brought in contact with a stomatal opening into which the water is quickly drawn by capillary action. Thus the germ tubes would build continuous water passages from the spores to the inner parenchyma of the leaf."

This apparent tendency of the germ tubes of Cercosporella Nesliae to grow towards the stomata of Neslia paniculata may possibly be a hydrotropic response occasioned by the moisture present in the stomatal cavities.

From 36-48 hours after the inoculation of the mustard leaves the germ tubes of the spores were noticed to have reached the stomata and to have entered the leaf through the stomatal pore (Fig. 8B). In this illustration the spores are depicted as lying in close proximity to the stomata, but where the spores are remote the germ tubes extend in a similar manner until penetration is effected through the stomatal pore.

From 72-96 hours after the inoculation of the leaves penetration through the stomatal pore is complete and the production of hyphae proceeds (Fig. 8C). The spore produces a septate, branching hypha which apparently must extend into the inner parenchyma of the leaf resulting in the production of disease symptoms, although the development of such hyphae cannot be traced by means of the method used in this phase of the investigation.

Entrance of the pathogene into the host appears to take place through the stomata of the lower epidermis as well as the upper epidermis.

In this study all of the diagrams were made with the aid of a camera lucida.

Fig. 8. Mode of penetration of the epidermis of Neslia paniculata by Cercosporella Nesliae.

The Relation of Humidity to Infection

Under the heading "proof of pathogenicity", it was stated that difficulty was encountered in establishing artificial infection in the greenhouse due to unfavourable humidity relationships. Successful infection was later established by placing the inoculated plants in a cheese-cloth cage which was kept moistened down at all times during the incubation period. To obtain some data on the percent relative humidity necessary for the establishment of infection, the relative humidity within the inoculation cage and in the greenhouse was determined by means of two accurately adjusted hygro-thermographs. Previous to making the determinations the hygro-thermographs were adjusted with the aid of a sling psychrometer and were standardized to give identical readings, and were then held under observation for several days before commencing the determinations.

These hygro-thermographs were then placed at the same height and at the same distance from the steam pipes, one being placed in the open greenhouse and one in the inoculation cage. The determinations were made over a period of one hundred and fifty-six hours, at the end of which period the readings at four hour intervals were taken from the charts. The averaged figures are given in Table 2. From the data given in this table it is seen that the mean percent relative humidity in the greenhouse over the given time interval was 50.3%, while the average relative humidity in the inoculation cage was 56.8% over the same time interval.

TABLE 2.

Percent relative humidity in the greenhouse and inoculation cage.

Hours	4	8	12	16	20	24	28	32	36	40	44	48	52	56	60	64	68	72
Rel. hum. in greenhouse	40	62	38	46	43	42	38	50	42	45	41	50	43	40	46	40	50	64
Rel. hum. in inoc. cage	58	80	50	48	64	48	42	52	44	44	80	72	48	52	44	45	84	83

Hours	76	80	84	88	92	96	100	104	108	112	116	120	124	128	132	136	140	144
Rel. hum. in greenhouse	58	48	41	42	48	72	51	46	39	40	36	44	56	56	50	43	40	52
Rel. hum. in inoc. cage	76	50	40	60	78	74	50	50	68	68	46	65	65	53	50	54	66	78

Hours	148	152	156
Rel. hum. in greenhouse	48	60	60
Rel. hum. in inoc. cage	61	64	62

Mean percent relative humidity in greenhouse = 50.3%

" " " " " inoc. cage = 56.8%

During this time interval the temperature in the greenhouse and in the inoculation cage fluctuated between 66-74°F., the mean temperature being 70°F.

At this time it should be pointed out that a relative humidity of 56.8% in the inoculation cage must be considered as the absolute minimum for successful infection as disease symptoms were only apparent when the inoculation cage was kept thoroughly moistened down at all times during the incubation period. It seems likely from greenhouse observations that the optimum relative humidity for the establishment of infection would be considerably higher than 56.8%.

In the summer of 1931 the disease of Neslia paniculata caused by Cercosporella Nesliae was extremely prevalent in the vicinity of Edmonton, and it is interesting to note that the mean relative humidities in percent in Edmonton during May, June, July and August of 1931 were respectively 65.1, 71.5, 67.4 and 70.0, which would indicate that the possibility of infection during May, June, July and August was present, which is in agreement with field observations previously discussed.

Pathological Histology

Methods

In this phase of the investigation a study was made of normal and diseased, leaf, stem and fruit-pedicel material. The diseased specimens were collected in the field, while the normal leaves and stems were obtained from plants growing in the greenhouse. The material was killed and fixed in medium-chromo-acetic fixing solution (2), washed in running water for 24 hours, and then imbedded in paraffin in the usual manner.

For paraffin sections made with the rotary microtome several combinations of stains were used with satisfactory results. An acid-fuchsin, light-green combination was found to be very useful. The sections were immersed in aqueous acid-fuchsin for 5 minutes, and in aqueous light-green for 10 minutes. They were washed between stains and differentiated in distilled water, and then mounted in gum-arabic-sugar mounting medium. This method gave a satisfactory differentiation, the diseased tissue and fungous mycelium staining pink to red, and the normal tissues staining blue.

Another stain which gave good results was Pianese III b (4), the normal tissues staining green, and diseased tissues and fungous mycelium staining a deep pink.

For the differentiation of lignified tissues and tracheal elements, crystal-violet and erythrosin as developed

by Jackson (9), gave excellent results, the lignified cells and xylem elements staining deep purple and the non-lignified cells light red, making a striking contrast. This combination was also found to be of value as a vital stain, the diseased tissue and fungous mycelium also staining a light red colour.

A thionin and orange "G" combination developed by Stoughton (16), was found to give excellent results as a vital stain with all material. Using this combination the parasite is stained violet-purple, cellulose walls yellow or green, and lignified tissues blue, making a very beautiful combination.

Lacto-phenol, cotton-blue stain also proved useful in staining freehand sections of fresh material.

In the presentation of the results of these histological studies, the terminology of Whetzel (18) has been used. The typical Cercosporella leaf lesion consists of a well defined inner region composed of broken down tissue, referred to by Whetzel as the "holonecrotic area or region". Surrounding this is a lighter coloured zone composed of cells which are diseased but still alive, this region being referred to by Whetzel as the "plesionecrotic zone".

The Leaf

In the healthy leaf of Neslia paniculata the upper and lower epidermi are composed of large, fairly regular, thick-walled cells, those of the upper epidermis

being slightly thicker-walled than those of the lower epidermis. The palisade parenchyma consists of a layer of elongated, loosely arranged cells lying immediately beneath the upper epidermis. The spongy parenchyma is composed of loosely arranged, more or less globose cells surrounded by large air spaces. The cells of the mesophyll contain a large number of chloroplasts which stain very deeply, sometimes making the interpretation of the sections difficult (Fig. 9).

The lesions vary greatly in size and often coalesce to form large necrotic areas. Each lesion is composed of a grey holonecrotic region surrounded by a light green to yellow plesionecrotic zone. The holonecrotic region consists of dead collapsed cells and is considerably thinner than the normal portion of the leaf. The epidermal and mesophyll cells are completely broken down and become replaced by masses of dark-staining residues. An abundance of intercellular mycelium is visible in this region (Fig. 10).

In the plesionecrotic zone the epidermal cells are normal in shape but the cells of the mesophyll are broken down and numerous intercellular hyphae are present and plainly visible due to the absence of the dark-staining material which is present in the holonecrotic region. Within the plesionecrotic zone, and in the immediate vicinity of the holonecrotic area, the cells of the mesophyll are greatly disorganized, but as one proceeds away from the centre of the lesion they gradually approach their normal

Fig. 9. Transverse section through a normal leaf of Neslia paniculata.

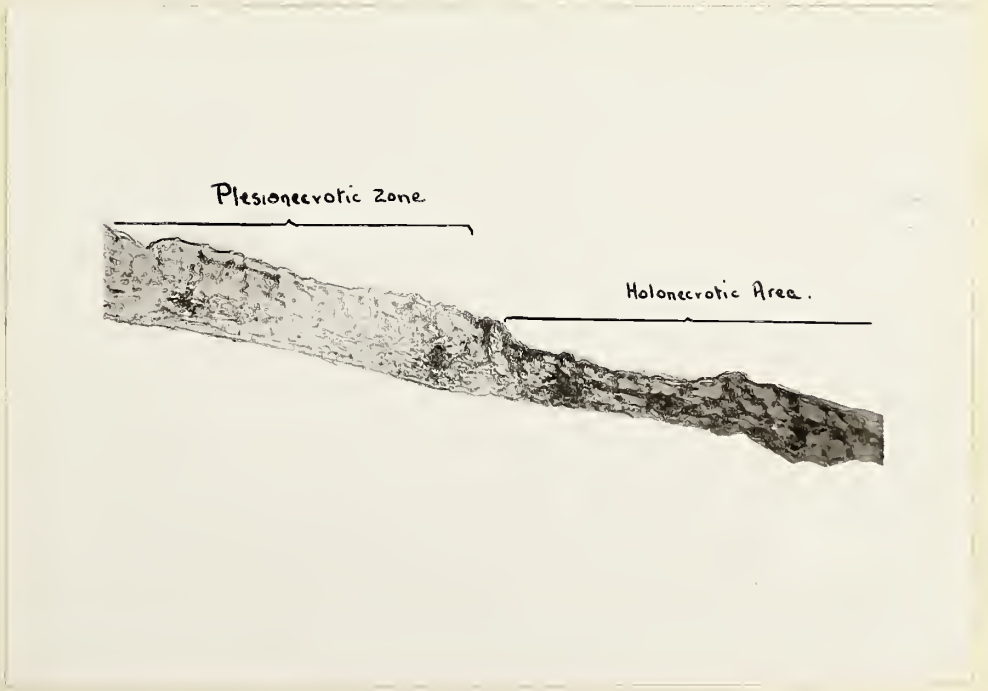

Fig. 10. Transverse section through a mature leaf lesion.

shape and arrangement. When the lesions are fairly large the intercellular hyphae may be observed in all parts of the leaf, but where the lesions are small and restricted the limit of the intercellular hyphae appears to be at the boundary of the plesionecrotic zone and the apparently normal tissues of the leaf.

In no case was evidence for "cicatrice formation" or the development of other "fungous barriers" obtained. The term "cicatrice" as used in this paper is applied to a band of healing tissue about the lesion or wound, the cells of which have been derived by division and enlargement of the mesophyll cells (18).

The fungous mycelium within the leaf is intercellular and appears to ramify unhindered through the tissues of the host, absorbing nutriment from the host cells and eventually bringing about their disintegration.

The Stem

The normal stem of Neslia paniculata consists of an epidermis of regular, thick-walled cells, within which is a fairly narrow cortex of parenchyma cells external to the inner parenchyma containing the vascular elements. The vascular elements are arranged in a discontinuous ring with typical endarch primary xylem and some secondary thickening. The secondary phloem is generally bounded by a layer of sclerenchyma cells, one to several layers thick, which may

or may not be continuous. The normal stem possesses a fairly extensive inner parenchyma with a wide pith of thin-walled cells. In older stems leaving a hollow pith (Fig. 11).

From a study of a transverse section of a diseased stem it is noticed that the pathogene is usually confined to the region of the outer cortex (Fig. 12), the cells of which are completely broken down and replaced by dark-staining masses identical with the diseased tissue of the leaf and partially composed of fungous mycelium. The epidermal cells are generally broken down but may persist in some cases.

While the fungous hyphae are generally confined to the tissues of the outer cortex a few hyphae were observed within the inner parenchyma. It would appear that the layer of thick-walled sclerenchyma cells bounding the secondary phloem forms at least a partial natural barrier to the advance of the parasite into the inner tissues of the host. This layer of sclerenchyma cells, however, is not continuous, which probably explains the occasional observation of hyphae within the inner parenchyma.

In the case of older stems, which have been diseased for some time, the pathogene proceeds to the formation of stromata in the outer cortical tissues. These dense cushions of fungous hyphae burst through the epidermis and appear externally as minute black dots (Fig. 13). The exact biological significance of these bodies cannot be

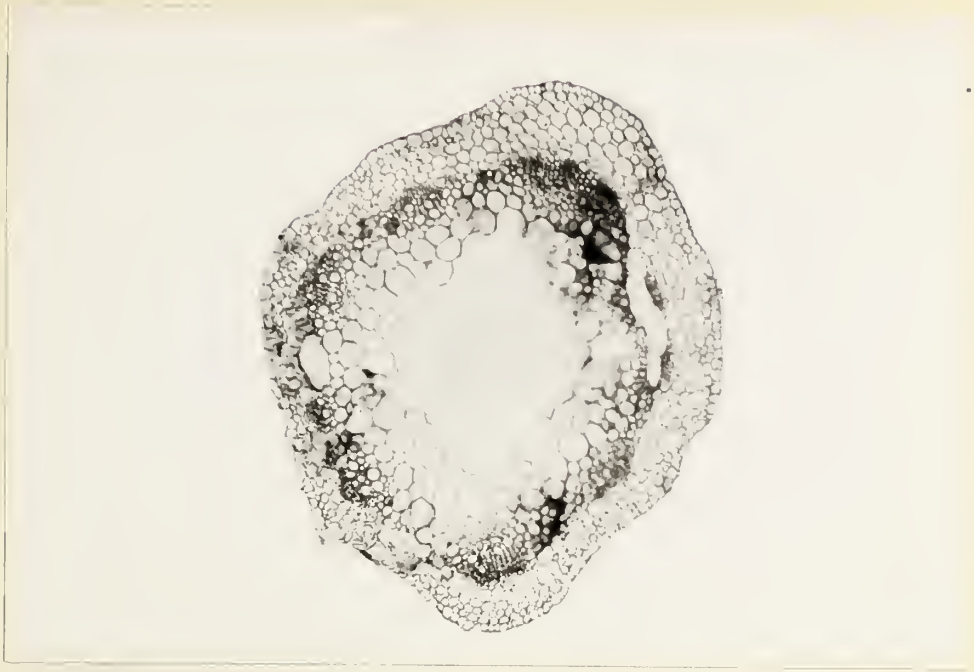

Fig. 11. Transverse section of a normal stem of Neslia paniculata.

Fig. 12. Transverse section of a diseased stem of Neslia paniculata.

definitely stated, but observations indicate that they form the foundation for the production of fruiting hyphae. Individual hyphae appear on the surfaces of these stromata (Fig. 13), but whether they are spore bearing hyphae or merely vegetative hyphae could not be definitely determined.

The Fruit Pedicels

The anatomy and histological changes in the fruit pedicels are essentially the same as those described for the diseased stems.

PHYSIOLOGICAL RELATIONSHIPS OF THE PATHOGENE

Cultural Characteristics

In the preliminary investigations the organism was noticed to be a very slow grower on ordinary potato dextrose agar. Hence, it was cultured on different media in an attempt to find a more suitable medium. Cultures on the different media were grown at room temperature and examined at the same age. In these studies the differences noted were largely in the size of the colony, size of the hyphal cells, pigment production in the medium and spore production. No striking morphological differences were noticed, and the growth on all media tried was relatively slow.

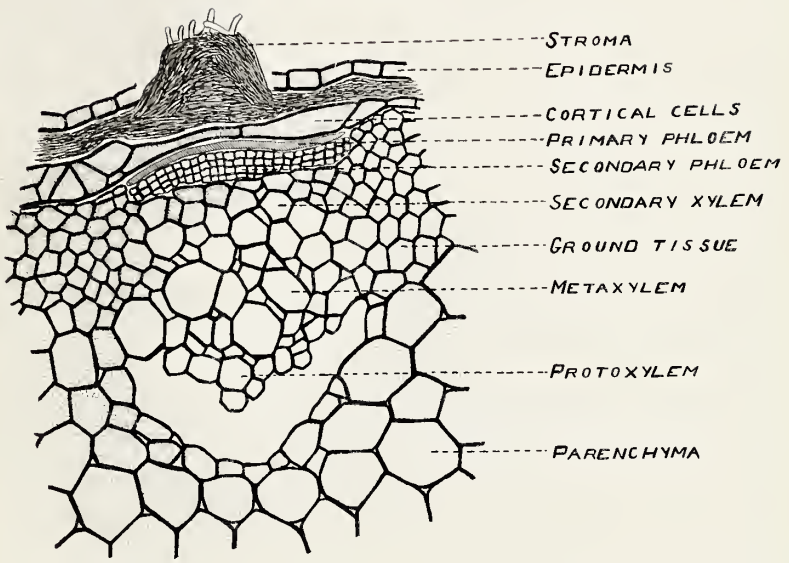

Fig. 13. Diagram of a transverse section of a diseased stem illustrating the nature of the stromata-like body.

On potato dextrose agar the colony appears as roughly circular, raised and olivaceous to olive-green turning black with age. After eight days the colony becomes surrounded with a fringe of mycelial growth, the surface being covered with a greyish aerial growth (Fig. 14). The mycelium is typical, 1-3 septate spores being produced only sparsely.

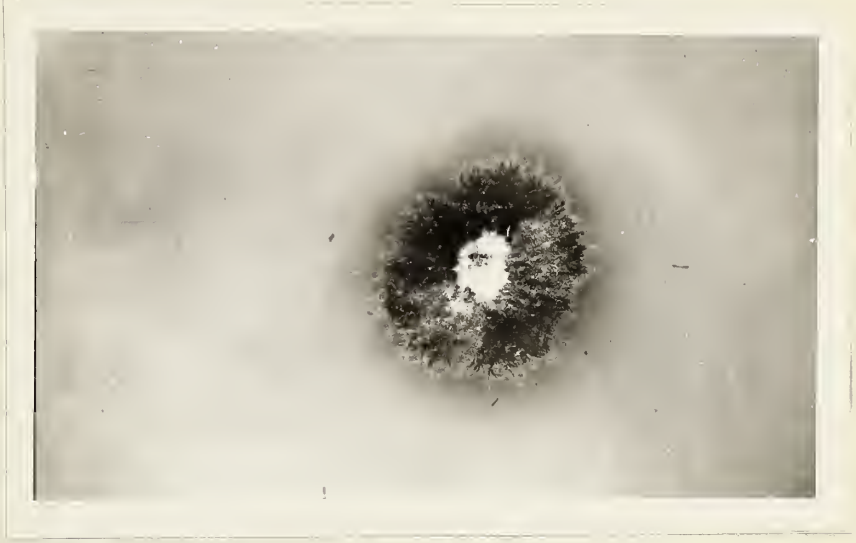

Fig. 14. Colony on potato dextrose agar.

On dehydrated potato dextrose agar the growth is fairly good, being faster than on ordinary potato dextrose agar but resembling the latter in most respects, the upper surface of the colony being irregular and the edge of the colony turning shiny-black. A slight red colour is imparted to the medium. The mycelium is filled with large oil globules, and the cells of the mycelial hyphae appear to be slightly larger than those of the hyphae growing on ordinary potato dextrose agar. The abundance of spore production appears to be affected but little.

On prune agar the colony appears as previously described, the main difference on this medium being the production of a pigment in the medium. The medium in the immediate vicinity of the colony turns an old-rose colour. The growth is a little slower than on potato dextrose agar and there is little or no spore production. The individual hyphae of the mycelium are not quite so stout as those on the two previously described media.

On corn meal agar the fungus grows considerably better than on the previously discussed media. The colony is less dense, and is composed of a matrix of loosely interwoven hyphae as compared with the solid, dense growth on most other media. In colour it is dark olive-green to dark-brown, the upper surface being composed of loose, wefty, greyish mycelial strands. The hyphal cells on this medium become swollen with stored food, as is the case on dehydrated potato dextrose agar, but no increase in the abundance of spore production was noticed. This medium appears to be suitable to a study of colony growth.

On sucrose-mustard agar. This medium was made up according to the following formula:

Agar	25.0 grams
Ball mustard tissue	500.0 "
Sucrose	2.0 "
Potassium nitrate	0.5 "
Water	1000.0 cc.

Green stems and leaves of ball mustard were extracted by heating for one hour in a steamer, after which the extract was filtered and the other ingredients added. The best growth of the organism was obtained on this medium which appears to be especially suitable for the measurement of colony growth. The colony is circular in outline but not dense, the under surface being olivaceous to dark-brown in colour and is surrounded by a lighter coloured mycelial fringe. The upper surface of the colony is watery in appearance and dark-brown to black in colour. A slight increase in the amount of spore production, compared with the growth on potato dextrose agar, was noticed.

Growth on boiled mustard stems. Green stems and leaves were removed from healthy plants in the field and 50.0 grams of this material were then weighed out into a 300 cc. Erlenmeyer flask and sterilized in the autoclave at 15 pounds pressure for 30 minutes. A small amount of the fungus from an agar culture was then added to each flask. After about two weeks incubation at room temperature a greyish-white, tufted, aerial mycelium makes its appearance on the surface of the boiled mustard leaves and stems in the flask. When examined more closely the mycelium is found to be identical with that occurring within the host plant, being thick-walled, hyaline, slightly granular, septate, and from 3.0-5.0 microns in width. When cultured as described above the organism produces the typical, elongate, 1-3 septate spores which are characteristic of

Cercosporella Nesliae. The fact that the organism will produce spores while growing as a saprophyte is noteworthy.

In Czapek's solution the organism grows in the form of small hard clumps composed of a mass of densely aggregated mycelium. These small clumps of mycelium range in diameter from 1-3 millimetres after two weeks incubation at room temperature, and are dark olive-green to grey in colour and generally aggregate into larger clumps. The mycelium is typical for the organism except that it is greener in colour, less branching, more septate, and presents a bead-like appearance with swollen cells. There is some spore formation but it is very sparse and the growth of the organism in this solution is extremely slow.

In Richard Duggar's solution the growth of the organism closely resembles that in Czapek's solution but is even slower.

The organism was also cultured on soil extract No. 7, peptone glucose agar No. 18 and on nitrate sucrose agar (7), but growth was extremely slow on these media.

Effect of the Reaction of the Substratum on the Vegetative Growth of C. Nesliae

The effect of hydrogen ion concentration on the growth of Cercosporella Nesliae was studied by culturing the organism on corn meal agar adjusted to different pH values. Bacto dehydrated corn meal agar was used in this

experiment and its pH was adjusted by the addition of varying amounts of approximately tenth normal sodium hydroxide and hydrochloric acid, the pH values being determined electrometrically. The pH values were calculated by means of the following formula:

$$pH = \frac{V + 0.4529 + 0.000026t}{0.00019832T}$$

Where V = observed potential in volts

t = temperature in degrees centigrade

= 40 C

T = 273.1 + t

= 273.1 + 40.0

= 313.1

After the pH value of the medium had been adjusted, 20 cc. lots were measured out into petri dishes and then resterilized in the autoclave, 12 plates of agar being prepared in this way for each medium. The hydrogen ion concentrations of the various media are given in Table 3, the values given representing the pH of the medium after two sterilizations for 30 minutes at 15 pounds pressure, occasioned by the necessity for resterilizing the agar after it had been pipetted out into petri dishes.

After the various media had been plated in the manner described a small piece of the fungous mycelium obtained from a pure agar culture was placed in the centre of each plate. The plates were then incubated at 20° C. At the end of 2 weeks incubation the average diameter of

TABLE 3.

Effect of hydrogen ion concentration on growth of Cercosporella Nesliae.

Medium	Reaction of medium		Reaction of non-inoculated medium after 2 weeks incubation		Reaction of inoculated medium after 2 weeks incubation		Average diameter of colony in millimetres
	E.M.F.	pH	E.M.F.	pH	E.M.F.	pH	
1.400 cc. agar + 52.3 cc. 0.1 N.NaOH	+0.078	8.6	--	-	-0.020	7.0	2.6
2.400 cc. agar + 28.6 cc. 0.1 N.NaOH	+0.053	8.2	-0.017	7.0	-0.023	6.9	3.9
3.400 cc. agar + 20.0 cc. 0.1 N.NaOH	+0.027	7.9	-0.036	6.7	-0.030	6.8	4.4
4.400 cc. agar + 11.4 cc. 0.1 N.NaOH	+0.018	7.0	-0.054	6.4	-0.035	6.7	5.7
5.400 cc. agar + 5.7 cc. 0.1 N.NaOH	-0.038	6.7	--	-	--	-	-
6.400 cc. agar + 0.0 cc. 0.1 N.NaOH	-0.057	6.4	-0.057	6.4	-0.057	6.4	9.3
7.400 cc. agar + 0.7 cc. 0.1 N. HCl	-0.065	6.2	-0.065	6.2	--	-	6.6
8.400 cc. agar + 1.4 cc. 0.1 N.HCl	-0.113	5.5	-0.104	5.6	-0.109	5.6	5.3
9.400 cc. agar + 2.8 cc. 0.1 N.HCl	-0.156	4.8	-0.157	5.0	-0.154	5.0	4.5
10.400 cc. agar + 4.3 cc. 0.1 N.HCl	-0.190	4.3	-0.187	4.2	-0.175	4.5	3.2

the colonies in millimetres was determined. The results, given in Table 3, represent the average diameter of at least ten measurements for each medium.

When the graph for growth as a function of hydrogen ion concentration is plotted from the values obtained it is seen that the optimum reaction for the growth of the organism is in the neighbourhood of pH 6.4. Growth falls away rapidly on both sides of the optimum, and it would appear that there is a very narrow pH range which is suitable to growth of Cercosporella Nesliae. With increase of either acidity or alkalinity the amount of growth decreases rapidly, the rate of decrease being roughly proportional on both sides of the optimum (Fig. 15).

Final Reaction of the Culture Medium

For a number of years it has been generally accepted that certain micro-organisms possess the ability to change the hydrogen-ion concentration of the medium upon which they are being cultured. Michaelis and Marcora (12) were probably the first to demonstrate that certain bacteria possess the ability to produce acid from carbohydrates and change thereby the colour of indicators present. These investigators in 1912 worked with Bacterium coli in lactose broth and showed that fermentation proceeded to a definite pH value and stopped, the initial pH of the medium having little influence. Sanford (13), working with

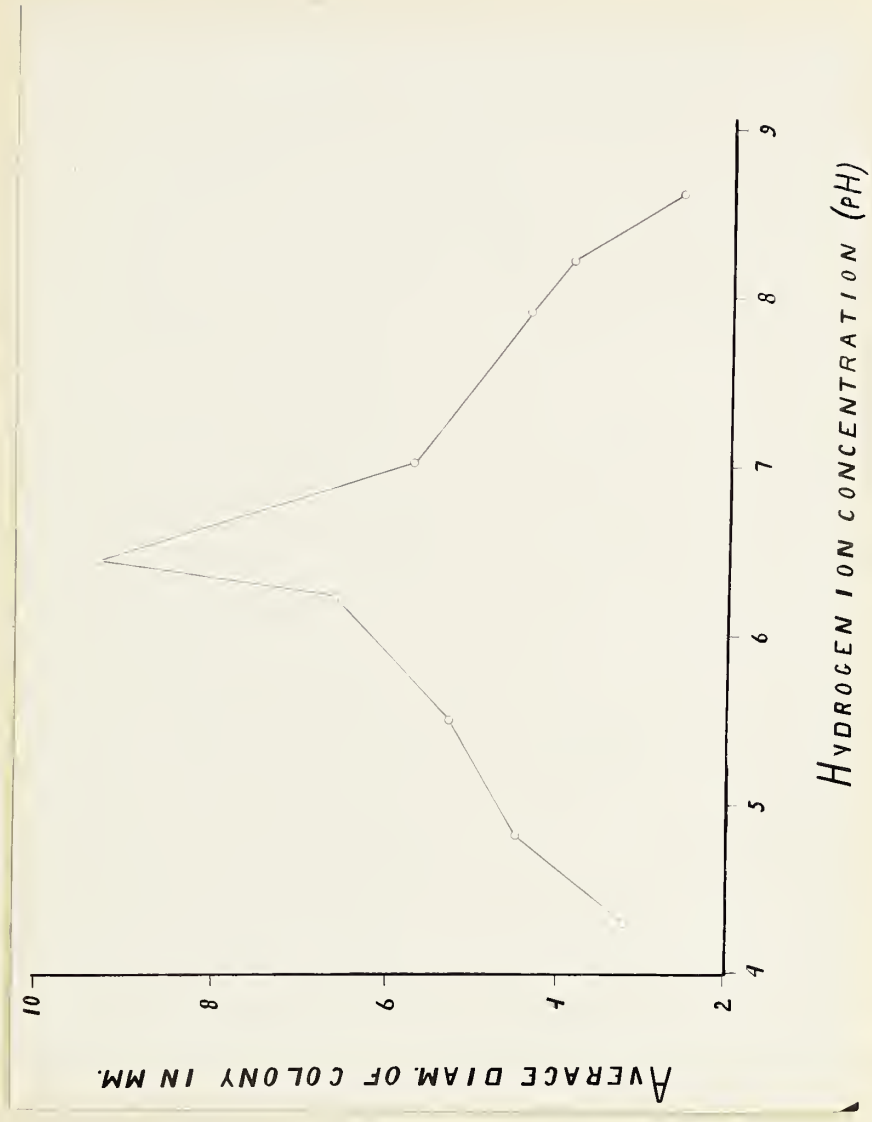

Fig. 15. Effect of hydrogen-ion concentration on the vegetative growth of Cercosporella Nesliae.

Actinomyces scabies, determined the effect of this organism on the reaction of Czapek's synthetic dextrose-nitrate solution. Electrometric determinations were made of the initial reactions of two series, with and without inoculum, determinations again being made for each series at ten day intervals for the acid media and forty-eight hours for the alkaline media. In every case it was noted that solutions containing inoculum soon became alkaline depending upon the rate of growth of the organism.

Sideris (15), working with Fusarium cromyophthorom, noted that the initial reaction of the culture medium was changed by the metabolic activities of the organism. The organism was cultured on a 2% dextrose solution, and also on an onion decoction, and the results obtained indicated that Fusarium cromyophthorom was capable of changing the initial reaction of its substratum by the liberation of either acidic or basic substances, this depending on the initial reaction of the solution. The results also indicated that the formation of acids may cease and alkalinity develop in media after all the sugar has been consumed by the organism. Klotz (10), working with Cercospora apii, noticed that the fungus failed to make appreciable growth at once at pH 4.0, 3.5 and pH 3.0, and later made appreciable growth at these concentrations and he states,

> "that this seems to be due to some change in the medium due either to the effects of the fungus or to the instability of the medium itself, or both."

In this investigation an experiment was planned to determine whether or not Cercosporella Nesliae was capable of changing the hydrogen-ion concentration of the culture medium. In the study of the effect of the reaction of the medium on the growth of the pathogene, plates of corn meal agar of various reactions were inoculated with the causal organism as previously described. At the same time, non-inoculated plates of the same series of media were incubated under the same conditions as the inoculated plates. At the end of two weeks incubation at 20°C. the colonies were removed from the centres of the inoculated plates and the reactions of the media from the inoculated and non-inoculated plates were determined electrometrically. The results obtained are given in Table 3. From these results it appears that the reaction of the media changed considerably with incubation, the change being greater on the alkaline side than on the acid side. The medium with an original pH of 8.2 dropped to pH 7.0 after two weeks incubation, the original pH of 7.9 dropped to pH 6.7, while the medium with an original pH of 4.8 increased to pH 5.0, there being some indication that the acid media tended to approach the optimum for the growth of the fungus, which remains constant throughout. From the results given in Table 3, and illustrated in Fig. 16, it is seen that the reactions of the inoculated and non-inoculated media at the conclusion of two weeks incubation are almost identical, the discrepancies between

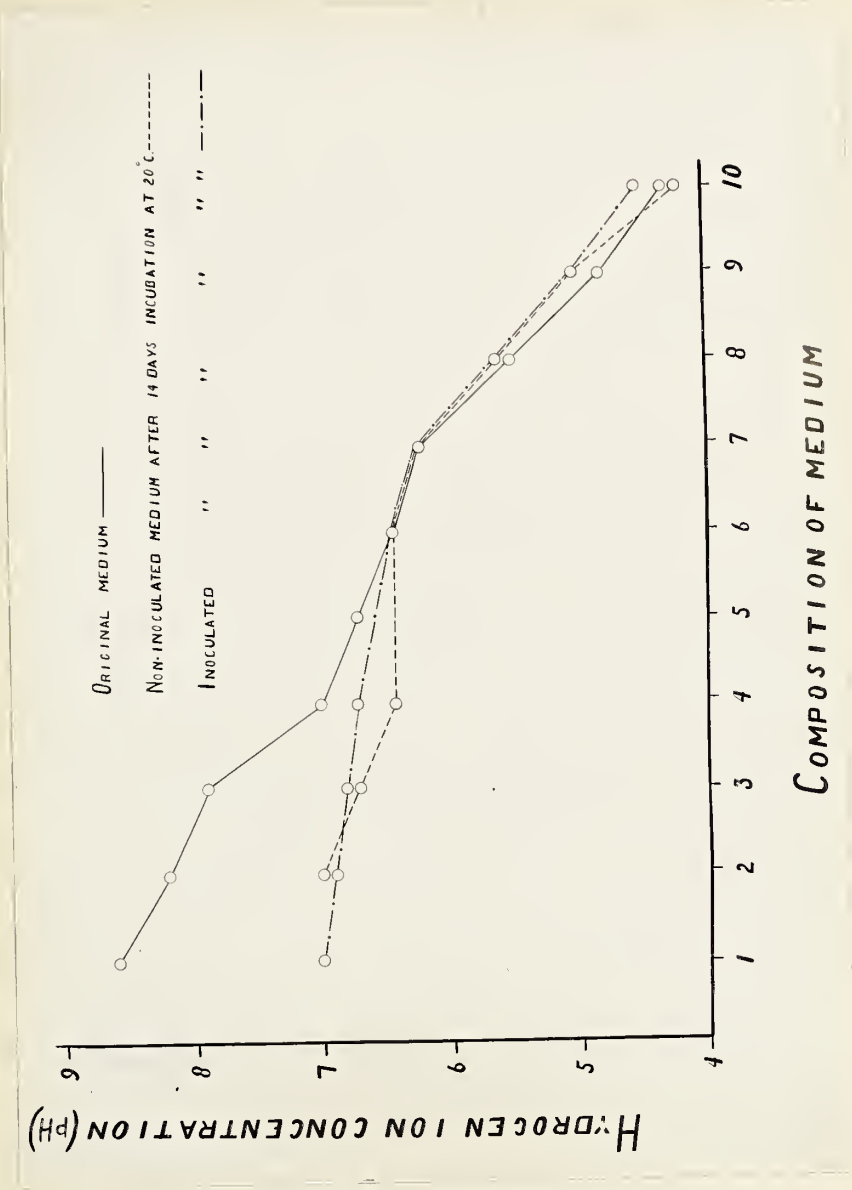

Fig. 16. Final reaction of the culture medium after 2 weeks incubation at 20°C.

the two sets of values doubtless being due to experimental error. From these results it was concluded that the change of reaction of the medium during incubation was due to instability of the medium itself rather than to the effects of the growth of Cercosporella Nesliae on the medium. However, should any change be effected by the fungus on the reaction of the medium, such change is probably localized in that portion of agar containing the colony which was removed from the centre of the plate.

The Effect of Hydrogen-ion Concentration on Spore Germination

The effect of hydrogen-ion concentration on the germination of spores of Cercosporella Nesliae was determined by means of the following procedure: the hydrogen-ion concentration of ordinary tap-water was adjusted to various pH values by the addition of varying amounts of 0.1 N NaOH, and 0.1 N HCl, the pH values being determined electrometrically as before. Eventually, a series of tap-water solutions was obtained ranging in hydrogen-ion concentration from pH 2.4 to pH 8.9. A concentrated suspension of spores was then made up in distilled water from a seven-day-old culture of the organism, and hanging drops of the various tap-water solutions, whose pH had been adjusted to the desired values, were inoculated with the spore suspension. The series was

incubated at 20°C., the hanging drops being made up in quadruplicate and the percentage germination was determined at 12 hour intervals for each hydrogen-ion concentration.

The results of this experiment are given in Table 4, and illustrated graphically in Fig. 17. From these results it is seen that at pH 2.4 the percentage germination is slight, with a gradual increase up to pH 3.7, followed by a steady rise as the neutrality point is approached, after which the percentage germination falls away rapidly again being very slight at pH 8.9.

After 24 hours incubation a marked increase was noted in the percentage germination, with a slight increase over this after 36 hours incubation, but the general trend of the curves is the same for the three time intervals. From the results given in Table 4, it is seen that the optimum pH for spore germination lies between pH 6.4 and pH 7.2, which agrees with the findings obtained in the experiment conducted on the effect of hydrogen-ion concentration on the vegetative growth of Cercosporella Nesliae, in which case the optimum was in the neighbourhood of pH 6.4. On the basic side of the neutrality point the percentage germination falls away very rapidly which further indicates that Cercosporella Nesliae is relatively intolerant to alkalinity.

Dosdall (6), working on the effect of hydrogen-ion concentration on the germination of spores of Helminthosporium sativum, noticed that optimum pH for

TABLE 4.

Spore germination at 20°C. of Cercosporella Nesliae in tap water of various hydrogen-ion concentrations.

pH	Hours	Percentage germination				
		a	b	c	d	Average
2.4	12	4.0	5.0	4.0	6.0	4.75
	24	10.0	8.0	10.0	13.0	10.30
	36	14.0	18.0	19.0	20.0	17.5
3.4	12	5.8	6.6	7.9	6.7	7.8
	24	27.0	26.0	29.0	25.0	26.8
	36	29.0	24.0	26.0	24.0	25.8
3.7	12	10.8	6.4	8.3	10.0	8.9
	24	37.0	34.0	29.0	31.0	32.8
	36	46.0	34.0	--	31.0	37.0
4.8	12	14.8	13.2	13.5	--	13.8
	24	42.0	39.0	35.0	--	38.6
	36	42.0	47.0	--	--	44.5
6.4	12	18.0	20.3	15.0	15.0	17.1
	24	44.0	46.0	53.0	36.0	44.8
	36	46.0	42.0	53.0	51.0	48.0
7.2	12	18.4	--	19.1	18.6	18.7
	24	36.0	--	51.0	50.0	45.7
	36	42.0	--	68.0	67.0	59.0
7.7	12	13.6	11.3	10.8	11.0	11.7
	24	35.0	45.0	40.0	41.0	40.3
	36	47.0	50.0	55.0	47.0	49.8
8.9	12	4.0	5.0	5.0	5.0	4.75
	24	-	15.0	14.0	-	14.5
	36	-	23.0	30.0	-	26.5

spore germination after 18 hours incubation at 19°C. was in the neighbourhood of pH 7.0, a second maximum being obtained at pH 9.2.

Webb (17), working with Fusarium sp., also obtained a bi-modal curve for the germination of Fusarium spores at various hydrogen-ion concentrations, and in this case the two maxima occurred at pH 2.8 and pH 7.4. A consideration of the graphs for the effect of hydrogen-ion concentration on the vegetative growth of Cercosporella Nesliae (Fig. 15), and for the effect of hydrogen-ion concentration on spore germination (Fig. 17), would appear to indicate that there is little possibility of the occurrence of a second maximum in the case of Cercosporella Nesliae.

Dosdall (6), and Webb (17), also noticed that the spores of Helminthosporium sativum and Fusarium sp. germinated over a wide range of pH values, which appears to be the case with the spores of Cercosporella Nesliae.

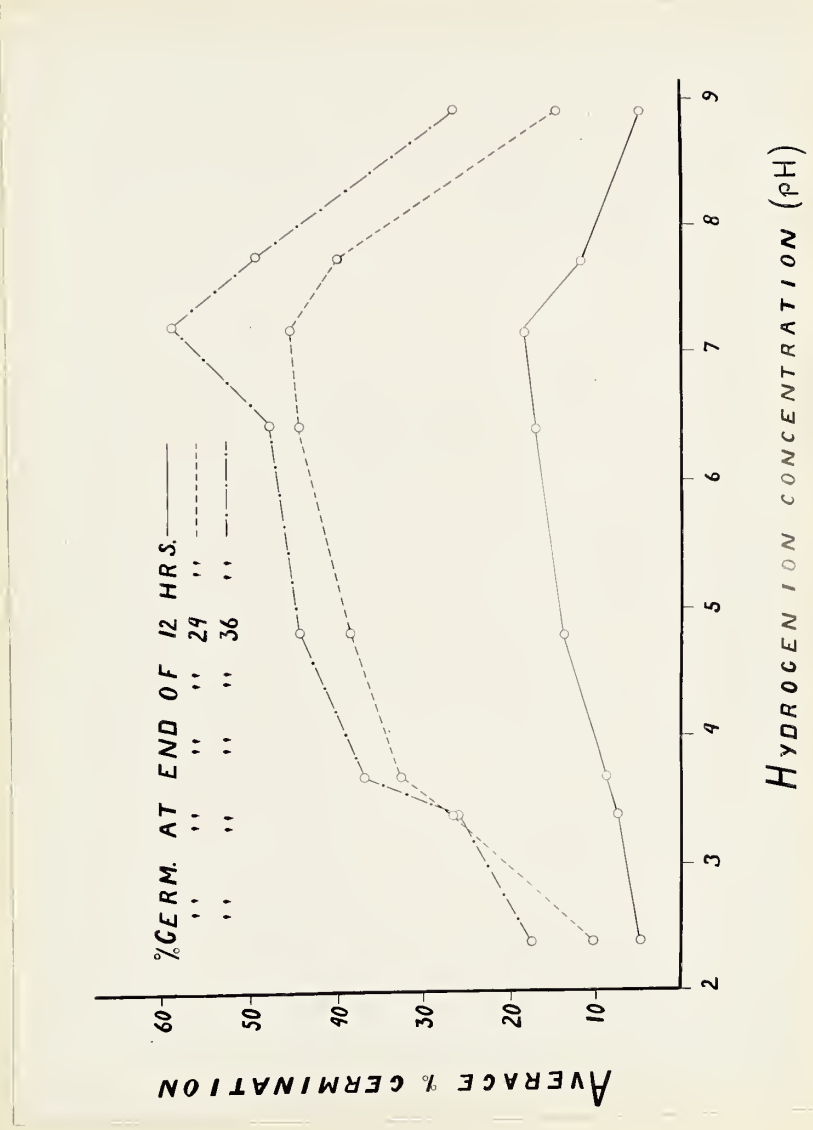

Fig. 17. Effect of hydrogen-ion concentration on the germination of spores of Cercosporella Nesliae.

Temperature Relationships of the Causal Organism in Culture

The effect of temperature on the vegetative growth of the organism in culture was studied by growing colonies on different kinds of agar at temperatures ranging from 5-25 degrees Centigrade. The cultures were prepared as follows:

The agar was melted in a hot-water bath and 10 cc. lots were measured out by means of a pipette into petri dishes. The petri dishes containing the agar were then sterilized in the autoclave and after they had been allowed to solidify each was inoculated with a small piece of fungous mycelium obtained from pure cultures of the organism. The organism was grown on potato dextrose agar, corn meal agar, and sucrose mustard agar, and was incubated at the five different temperatures of 5, 10, 15, 20 and 25 degrees Centigrade. The inoculated plates were incubated for a period of two weeks, when the colonies were measured. The results obtained in this experiment are given below in Table 5 and are presented graphically in Fig. 18, each value in the table representing the average diameter of twenty colonies. It will be noted that the maximum radial growth

TABLE 5.

Vegetative growth of Cercosporella Nesliae at different temperatures.

Medium	Average diameter of colony in millimetres at different temperatures Centigrade				
	5	10	15	20	25
Corn meal agar	2.0	3.2	5.3	11.4	8.9
Sucrose mustard agar	2.3	3.9	6.8	11.0	7.1
Potato dextrose agar	2.0	3.2	8.1	8.5	4.8

of the organism was attained in the neighborhood of 20°C., on the three media used. At the higher temperatures the vegetative growth of the organism was much slower, the colony appearing as a hard, constricted, knob-like mass. The colonies incubated at 30°C. were too small to measure accurately due to their restricted nature. From the data presented in Table 5 it is apparent that the optimum temperature for the growth of Cercosporella Nesliae in culture is approximately 20°C.

Resistance to Desiccation

To obtain some idea of the resistance of the pathogene to dry conditions, the following experiment was outlined. A concentrated suspension of spores of

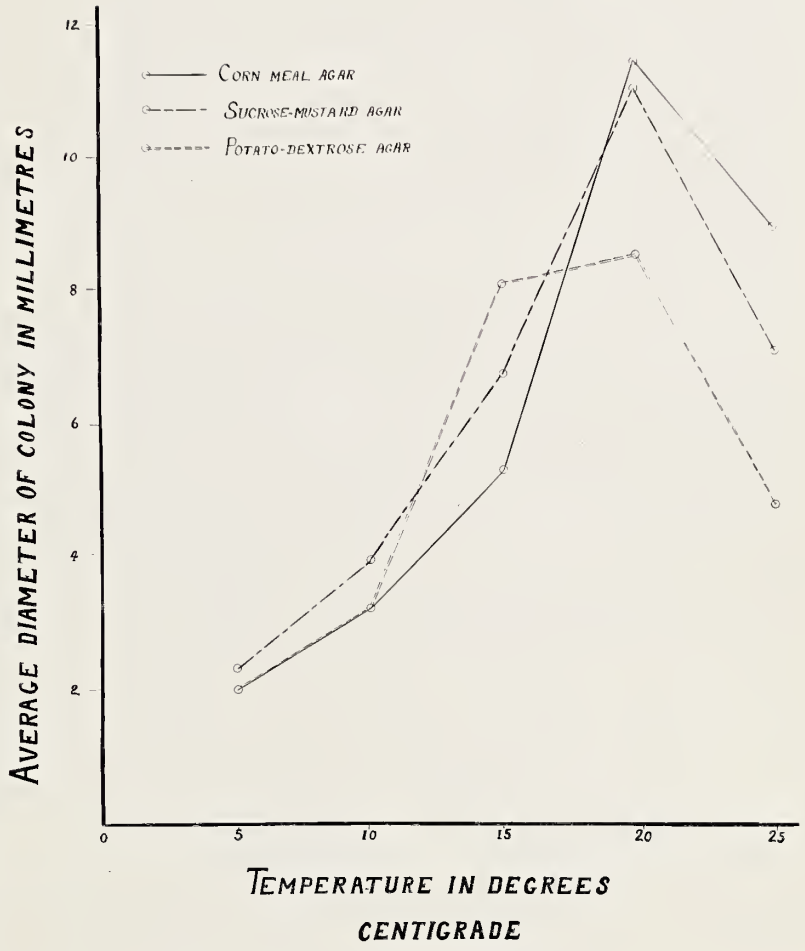

Fig. 18. The effect of temperature on the radial growth of Cercosporella Nesliae on different media.

Cercosporella Nesliae, obtained from pure agar cultures of the organism, was made up in sterile water, and using aseptic precautions a number of sterile cover glasses were covered with the suspension so as to form a thin film over their surface. These were then placed in sterile petri dishes. Duplicate series of these cover-glasses were made and the petri dishes containing them were placed in a desiccator over calcium chloride, and desiccation allowed to proceed at room temperature.

From time to time cover-glasses were removed from each series and were planted on corn meal agar slants. The results obtained in this experiment are given in Table 6, positive growth being indicated by (+) and negative growth by (-).

TABLE 6.

Resistance of spores of Cercosporella Nesliae to desiccation.

Glass surface	Period of desiccation in days							
	4		18		42		62	
	A	B	A	B	A	B	A	B
Series "A"	+	+	+	+	+	+	-	+
Series "B"	+	+	+	+	+	+	-	-

The fact that the spores of the causal organism, when subjected to the severe conditions of desiccation mentioned, still remained viable after 42 days

desiccation would indicate that the pathogene is quite resistant to desiccation.

Klotz (10), working with Cercospora apii, inoculated sterile cover-glasses with a spore-mycelium suspension of the fungus, placed them in petri dishes and allowed desiccation to proceed at room temperature. Positive growth was still obtained after 68 days desiccation demonstrating the resistance of that organism to desiccation when less protected. Klotz also studied the effect of drying on the germination of conidia of Cercospora apii, by placing blighted celery leaves in desiccators, and removing spores from time to time and testing their germination in tap-water. Klotz found that spores so treated still retained their viability after 170 days desiccation at room temperature.

To obtain data on the effect of drying on the viability of Cercosporella Nesliae under more natural conditions, plantings on corn meal agar were made from herbarium material collected some months previously. The herbarium specimens had been preserved in paper folders, and had been allowed to dry under ordinary laboratory conditions, the relative humidity in the laboratory ranging from 20-25% during this period. The results of these isolation experiments are given in Table 7.

TABLE 7.

The effect of desiccation of dried herbarium material on the viability of Cercosporella Nesliae.

Date of isolation.....February 29, 1932.				
No.	Material	Stage of disease	Date of collection	Growth
1	Leaf	Leaf lesions	July 9, 1930	Positive
2	Stem	Lesions confined	July 20, 1930	Positive
3	Stem	Stromata developed	Aug. 8, 1930	Positive
4	Leaf	Leaf lesions	June 25, 1931	Positive
5	Stem	Lesions confined	Aug. 15, 1931	Positive
6	Stem	Stromata developed	Aug. 30, 1931	Positive

From the data given in Table 7 it may be observed that successful isolations were made from all available herbarium material, including leaf material which had been collected 19 months previous to the date of isolation. These results further indicate that the pathogene within the diseased host material may retain its viability for a considerable period of time.

ECONOMIC SIGNIFICANCE OF *CERCOSPORELLA NESLIAE*

Cercosporella Nesliae causes considerable damage to ball mustard plants in the field. Infected plants are noticeably less vigorous than healthy plants, being stunted and generally of a pale green colour, and present a "dried-up" appearance as compared with the sturdy, dark green, turgid condition of healthy plants. This is probably due to a reduction in photosynthetic activity occasioned by the development of leaf lesions and the subsequent fall of diseased leaves.

Field observations indicate that the total amount of seed produced by infected plants is considerably less than is the case with healthy plants. Healthy plants normally produce an abundance of seed, while diseased plants, on the other hand, appear to lack the necessary vigour to produce the normal amount of seeds, and such seed that is produced is generally greatly reduced in size.

Ball mustard plants grown in the greenhouse from seeds obtained from diseased plants failed to develop the disease symptoms when placed under optimum conditions. Plantings were made of seedlings on corn meal agar immediately after they had emerged, but in no case was the organism isolated. These facts would indicate that *Cercosporella Nesliae* does not overwinter within diseased seeds or on the surface of such seeds.

Cross-inoculation experiments using several species of mustards and some of the more common of the native weed species were made to determine the host range of Cercosporella Nesliae. The following weed species were inoculated:

Tower mustard (Arabis glabra), Tumbling mustard (Sisymbrium altissimum), Tansy mustard (Sisymbrium incisum), Indian mustard (Brassica juncea), Wormseed mustard (Erysimum cheiranthoides), Hare's ear mustard (Conringia orientalis), Stinkweed (Thlaspi arvense), Shepherd's purse (Capsella bursa-pastoris), and Western false flax (Camelina sativa). All of the above hosts were found to be immune to Cercosporella Nesliae under optimum conditions for the establishment of infection in the greenhouse.

In the summer of 1931 a number of plots of ball mustard were badly infected in the field, and in the immediate vicinity of these plots the following vegetable crops were growing:

Lettuce, turnips, radish, spinach, carrots and garden beets. All of these vegetables failed to develop symptoms of the disease. The above facts indicate that Cercosporella Nesliae must be very specific as to its host, and as a menace to economic crop plants cannot be considered as important.

SUMMARY

1. Cercosporella Nesliae is a pathogene which causes a disease of ball mustard (Neslia paniculata) in western Canada. The disease was noticed to be prevalent throughout the province of Alberta in all districts where ball mustard plants occurred.

2. The pathogene attacks the leaves causing the development of well defined lesions. Two to three weeks later, lesions appear on the Stems and gradually spread until the whole stem may become involved. In advanced stages of the disease the fruit pedicels and the fruits may turn black, badly infected plants in the field presenting a characteristic blackened and stunted appearance.

3. The pathogenicity of Cercosporella Nesliae was demonstrated by artificial inoculations of ball mustard plants. Successful infection was obtained only when a fairly high relative humidity was maintained.

4. The morphology of the organism in general agrees with the mycological description for the genus. Stromata-like bodies, composed of dense cushions of fungous mycelium, burst through the epidermis and appear on the surface of diseased stems and fruit pedicels, and in some cases on the surfaces of the leaf lesions.

5. Cercosporella is a genus of the Fungi Imperfecti, belonging to the order Moniliales and the family Moniliaceae.

6. Cercosporella Nesliae appears to overwinter in the form of dormant mycelium within the diseased stems and other diseased parts of dead plants. Spore production commences in the spring and results in the establishment of primary infection within the leaves. The stem lesions appear to develop as a result of secondary infections by conidia produced from the leaf lesions. Secondary infection takes place from time to time throughout the summer, the fungus once more entering the dormant state with the return of cool weather in the fall, in which state it overwinters.

7. Penetration of the leaves is effected through the stomata, the spores exhibiting a tendency to produce germ tubes from those portions lying in closest proximity to the stomata. This would appear to be a hydrotropic response.

8. Greenhouse experiments indicate that a relative humidity of 57% should be considered as the minimum for the establishment of infection.

9. Within the host the pathogene causes a breaking down of the tissues, these tissues being replaced by masses of dark-staining material partially composed of fungous mycelium. No evidence for "cicatrice formation", or the development of other "fungus barriers" was observed in any of the material studied.

10. The organism grows very slowly in artificial culture. It was successfully cultured on sterilized stems and leaves of healthy ball mustard plants.

11. The hydrogen-ion concentration of the culture medium affects the vegetative growth of the organism considerably. The optimum hydrogen-ion concentration for the growth of the organism is in the neighborhood of pH 6.4. The organism appears to lack the ability to change the reaction of the culture medium, the change in reaction of the medium being due to the instability of the medium itself rather than to the action of the fungus growing upon it. Maximum spore germination occurred in the neighborhood of pH 7.0.

12. The optimum temperature for the vegetative growth of Cercosporella Nesliae in culture is approximately 20°C.

13. The organism is quite resistant to desiccation, successful isolations being made from herbarium material collected 19 months previously.

14. Cercosporella Nesliae causes a reduction in vigour of infected plants, and a reduction in seed yield. Cross-inoculation experiments using several of the more common weed species were unsuccessful. Several of the more common vegetable crops are apparently immune to the pathogene, indicating that as a menace to economic crop plants, Cercosporella Nesliae cannot be considered important.

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